Chemistry & Biology
Review

Clinical Grade iPS Cells: Need for Versatile Small Molecules and Optimal Cell Sources

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http://dx.doi.org/10.1016/j.chembiol.2013.09.016

Adult mammals possess limited ability to regenerate their lost tissues or organs. The epoch-making strategy of inducing pluripotency in somatic cells incorporates multiple applications in regenerative medicine. However, concerns about the clinical translation of induced pluripotent stem (iPS) cells still exist because of the occurrence of aberrancies, even in genome integration-free methods. As cellular reprogramming is multi-gene-oriented, versatile, bioactive small molecules could concomitantly modulate the transcriptional machinery and aid the generation of clinical grade iPS cells. The availability of optimal cell sources has additional influence on the clinical translation of iPS cells. Herein we provide a critical overview of methods and cell sources available for iPS cell production. We think the review will be a useful resource for researchers who aim to develop small molecules for speeding up the journey of iPS cells from the laboratory to the clinic.

Cellular regeneration is a widely variable biological feature of all living organisms. In humans, who inherently lack the capacity to regenerate lost tissues or organs, the physical plasticity of embryonic stem cells (ESCs) and their ability to generate the desired cell types under culture conditions offer potential clinical solutions (Evans and Kaufman, 1981). However, ESC-derived cells may lead to immune-mediated rejection because the antigens expressed in the recipient patients are different. An alternative method, termed *somatic cell nuclear transfer*, or *SCNT*, uses patient-derived stem cells that are genetically identical to the donor nucleus. Gurdon successfully demonstrated this technique in frogs (Gurdon, 1962), which changed the scientific view that the fate of the specialized cells is irreversible, and Wilmut and colleagues substantiated the potential of this technique by cloning a mammal (Wilmut et al., 1997). However, attempts to use this technique in humans have only recently met with success after the development of refined protocols that circumvent major roadblocks, such as early embryonic arrest and suboptimal activation of key factors (Tachibana et al., 2013). Another issue in ESC research is that it is ethically controversial because experimental use of an early-stage embryo could potentially cause a human life to be destroyed.

About five decades later, on the basis of early groundbreaking work, Takahashi and Yamanaka (2006) provided a solution to the sourcing issues associated with ESCs by generating induced pluripotent stem (iPS) cells via the forced expression of four transcription factors. This discovery was proclaimed to have the potential to ease the controversy and ethical dilemmas associated with ESCs and to effectively bypass the problem of immune rejection. Consequently, the two pioneers of cellular reprogramming, Dr. John Gurdon and Dr. Shinya Yamanaka, won the 2012 Nobel Prize in Physiology or Medicine. Despite the exciting progress made under laboratory conditions, several barriers still hinder the transfer of iPS cell technology to the clinic. For example, there is increasing awareness of the similarities between iPS cells and cancer cells, and the genetic methods used to generate iPS cells can be a huge setback during evaluation of study protocols by international regulatory bodies (Barrilleaux and Knoepfler, 2011). The current iPS cell-engineering process may produce cells of variable quality (Gore et al., 2011; Kim et al., 2010). Similarly to any other product, cost and the time consumed are also significant concerns for the generation and marketing of clinical grade iPS cells (CGiPS) (Barrilleaux and Knoepfler, 2011). It may take months to validate and differentiate iPS cells prior to their clinical use. Hence, these cells are probably not ideal for the most acute and/or life-threatening illnesses and injuries that require immediate treatment. Current batch-prepared ESC-based products can be cheaper than iPS cells and are readily available. Hence, the expectation that iPS cells will be phased out and will replace ESCs has yet to be realized.

In this regard, the rapid generation of iPS cells using a combination of small molecules has attracted immense interest because this approach would eliminate the risk of genetic modification (Pandian et al., 2012a). Experimental practice thus far suggests that small molecules are easy to handle and could be readily available. Also, small molecules may have little or no side effects after the desired cellular phenotype is achieved. However, chemical reprogramming approaches may also introduce genetic or epigenetic abnormalities into the resultant iPS

cells. The directed targeting of desired genes in the body is not straightforward, owing to the dynamic epigenome. Thus, there is a demand to design and synthesize tailor-made small molecules with the specific mechanism of action, such as effect on epigenetic activity, to control the complicated gene networks associated with pluripotency. Also, the choice of cell source plays a pivotal role in the development of appropriate small molecules to generate CGiPS. Herein we give an overview of the various cell sources and reprogramming protocols available for deriving iPS cells with emphasis on cellular reprogramming with small molecules alone. The challenges and opportunities of developing innovative strategies that could potentially send the somatic cells back in time to an embryonic (pluripotent) state and/or push them into totally new types of cells are also briefly discussed.

Routes to the Efficient Induction of Pluripotency in Somatic Cells

The scientific principles underlying cellular reprogramming and the technologies associated with it have been under development for the past six decades. Some of the early successes include demonstration that a nucleus can be isolated and transplanted from late-stage embryos into enucleated oocytes (Briggs and King, 1952), establishment of immortal pluripotent cell lines from tumors of germ cell origin (Kleinsmith and Pierce, 1964), isolation of pluripotent cells directly from the embryo (Evans and Kaufman, 1981), and production of myofibers from fibroblast cell lines infected with retroviral vectors harboring the skeletal muscle factor MyoD (Davis et al., 1987). Some of these landmark early experiments provided a platform that inspired Takahashi and Yamanaka to screen for and identify four reprogramming factors in mouse fibroblasts (Oct-3/4, Sox2, Klf4, and c-Myc) that are essential for ESCs (Takahashi and Yamanaka, 2006). A milestone was later reached when iPS cells derived from human somatic cells were established (Takahashi et al., 2007; Yu et al., 2007). The iPS cells producing high levels of c-Myc protein often lead to the development of tumors, probably because c-Myc encourages not only self-renewal but also tumorigenesis. A subsequent study showed that pluripotency could be conferred on differentiated mouse and human cells without c-Myc (Nakagawa et al., 2008), but with low induction efficiency. The risk of tumor formation led researchers to develop integration-free strategies by employing adenoviruses, Sendai virus, plasmids, and protein-based protocols (Ban et al., 2011; Kim et al., 2008, 2009; Maherali et al., 2007; Mali et al., 2008; Mathieu et al., 2013; Okita et al., 2007, 2008; Park et al., 2008; Seki et al., 2010; Shu et al., 2013; Si-Tayeb et al., 2010; Stadtfeld et al., 2008; Zhang et al., 2011). Nevertheless, induction efficiency had to be compromised in these nonintegrative methods.

Rossi and colleagues developed a better approach to reprogramming human fibroblasts into iPS cells using a synthetic mRNA (Warren et al., 2010). This strategy is superior to virus-mediated gene transfer protocols regarding both conversion efficiency and kinetics. A miRNA-based strategy successfully reprogrammed mouse and human somatic cells to pluripotent cells (Anokye-Danso et al., 2011). The PiggyBac Transposon Vector–based method (System Biosciences, Mountain View, CA) is another zero-footprint method for iPS cell generation (Woltjen et al., 2009). The popular nonintegrating methods, such as the episomal plasmid-based method and the Sendai virus-based method are still highly favored to reprogram different cell types (Ban et al., 2011; Okita et al., 2011; Yu et al., 2009). Synthetic mRNA-based protocols have maximum efficiency (about 36-fold) over the original retrovirus-mediated system for delivering reprogramming factors. However, the lability of RNA and the undesired induction of innate antiviral defense pathways remain a barrier to their clinical use. Hence, there is a need to improve the established protocols and/or to develop clinically friendly strategies for achieving CGiPS. Cellular reprogramming with chemicals alone is a strategy thought to have better clinical prospects because it avoids genetic manipulation and could be readily available. Currently available reprogramming protocols to achieve usable iPS cell lines have their own induction efficiency and time frame advantages and disadvantages (Table 1). Numerous reports are now available on better ways to generate clinically useful iPS cells from various cell sources (Loh et al., 2009) and associated mechanisms (Gutierrez-Aranda et al., 2010; Lee et al., 2012; Lu et al., 2012), with remarkable progress made over a short period (Figure 1).

Large Impact of Small Molecules in the Generation of iPS Cells

Small molecules have already been successfully employed in the generation of iPS cells. Shi and colleagues identified small molecules that could enhance the reprogramming efficiency of the genetic approach in mouse cells (Shi et al., 2008a). Dual inhibition (2i) of the signaling pathways (MEK and GSK3) reduced the time frame required for reprogramming mouse embryonic fibroblasts (MEFs) and neural precursors into pluripotent cells (Silva et al., 2008) even in the absence of Sox2 and c-Myc. Likewise, small molecules that inhibit the signaling factors associated with differentiation could either enhance the efficiency of iPS cell generation or substitute one or more reprogramming factors in mouse cells (Lyssiotis et al., 2009; Pandian and Sugiyama, 2012; Shi et al., 2008a, 2008b; Yuan et al., 2011). Identification of an Oct-3/4 activator (OAC1) in MEFs suggested that even Oct-3/4 could be substituted (Li et al., 2012). Small-molecule-mediated inhibition of mammalian target of rapamycin facilitated the reprogramming of MEFs into iPS cells (Chen et al., 2011). Two-factor reprogramming of human neural stem cells into pluripotency could be achieved with forskolin, a diterpene activator of adenylate cyclase (Hester et al., 2009).

Dynamic chromatin modifications facilitate the establishment and maintenance of pluripotency in somatic cells. A combination of the G9a histone methyltransferase inhibitor BIX-01294 and the L-channel calcium agonist Bayk8644 artificially induced pluripotency in the absence of Sox2 and c-Myc (Mikkelsen et al., 2008). Small-molecule inhibitors of chromatin-modifying enzymes such as DNA methyltransferase and histone deacetylase (HDAC) improved reprogramming efficiency by inducing changes in global-level changes in gene expression (Li et al., 2009; Lin et al., 2009; Mali et al., 2010). HDAC inhibitors, such as suberoylanilide hydroxamic acid (SAHA) and trichostatin A, increase the SCNT-mediated reprogramming efficiency by about 5-fold in a concentration- and time-dependent manner (Kishigami et al., 2006). Among the several small-molecule

Table 1. Comparison of Various Methods to Generate iPS Cells

Reprogramming Methods	Cells	Efficiency (%)	Time to Generate Usable iPS Cell Line	Major Drawback	Cost	Clinical Safety	References
Integrating methods							
Virus-based	MEF	0.01	~4 weeks	teratoma formation	medium	medium	Takahashi and Yamanaka, 2006; Takahashi et al., 2007
Retrovirus	HDF						
Lentivirus	MEF, FF	0.001–0.01				low	Yu et al., 2007; Hotta et al., 2009
Non-virus-based Transposons	MEF, HDF	0.01	~3 weeks	low efficiency	medium	medium	Woltjen et al., 2009
Nonintegrating methods							
Virus-based							
Adenovirus	TTF, fetal liver	0.0001, 0.0006	~30 days	low efficiency	high	medium	Stadtfeld et al., 2008
Sendai virus	CB cells	0.01	~28 days	low efficiency	high	medium	Ban et al., 2011
Non-virus-based							
Protein	HNF	0.001	~56 days	slow process	high	medium	Kim et al., 2009
mRNA	BJ fibroblasts	>1	~18 days	stability and trigger innate antiviral defense pathway	high	medium	Warren et al., 2010
miRNA	BJ fibroblasts	0.02−1	14–18 days	possibility of integrated vector, subfragments	high	medium	Anokye-Danso et al., 2011
Episomal vectors	foreskin fibroblasts	0.01–1	17–21 days		high	medium	Yu et al., 2009; Okita et al., 2011
Combined small-molecule approach							
OSKM+PD0325901 + CHIR99021	mNSC	0.11	30 to ~40 days	problems associated with the presence of retrovirus	medium	low	Silva et al., 2008
O+NaB+PS48+A-83-01+PD0325901	NHEK	0.00004					Zhu et al., 2010
O+A-83-01+AMI-5	MEF	0.02					Yuan et al., 2011
OS+VPA	HDF	0.5					Huangfu et al., 2008
Chemicals alone	MEF	0.2	40 days	not determined	low	high	Hou et al., 2013

CB, cord blood; FF, fetal fibroblast; HDF, human dermal fibroblast; HNF, human neonatal fibroblast; K, Klf4; M, c-Myc; MEF, mouse embryonic fibroblast; NaB, sodium butyrate; NHEK, normal human epidermal keratinocyte; mNSC, mouse neural stem cell; O, Oct4; S, Sox2; TTF, tail-tip fibroblast; VPA, valproic acid.

inhibitors that induce changes in epigenetic modifications, HDAC-inhibiting valproic acid and sodium butyrate led to a dramatic increase in reprogramming efficiency (about 100-fold) (Liang et al., 2010; Shi et al., 2008a) (Figure 2A). Ubiquitously used chemicals enhanced the generation of both mouse and human iPS cells (vitamin C; Esteban et al., 2010) and the reprogramming efficiency of MEFs through hyperosmosis, which reduced global demethylation level (sodium chloride; Xu et al., 2013). The chemical element lithium demonstrated a surprising effect of enhancing the reprogramming efficiency of both MEFs and human umbilical vein endothelial cells (HUVECs) into iPS cells (Wang et al., 2011a). Induction of pluripotency using only a single gene and a combination of small molecules in mouse and human cells has brought us a step closer to cellular reprogramming with small molecules alone (Li et al., 2011; Zhu et al., 2010). Deng and colleagues finally accomplished this complex feat by using seven small molecules to generate chemically induced iPS cells (CiPS) cells from mouse somatic cells with an induction efficiency of about 0.2% (Hou et al., 2013). We think that generating CiPS cells from human somatic cells will be achieved in the near future through the identification of small molecules that activate key developmental factors such as OCT4. Recent progress suggests that a number of chemicals are capable of enhancing reprogramming efficiency of fibroblasts into pluripotent stem cells (Figure 2B, Table 2), suggesting that there is significant room for improvement in the current protocols for and optimization of complete chemical reprogramming.

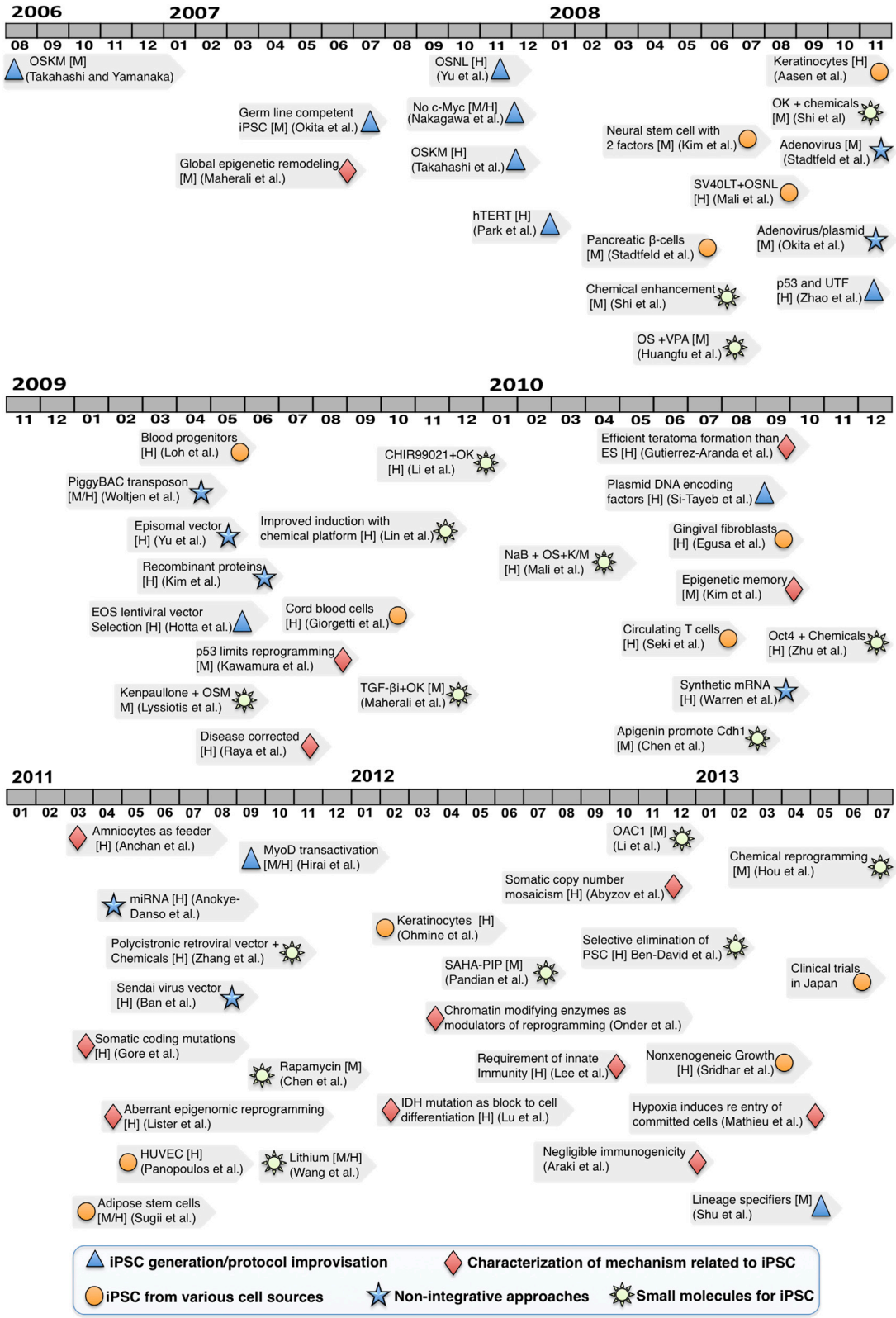


Figure 1. A Graphical Overview of Some Hallmark Studies of iPS Cells Published between 2006 and July 2013 Carried Out in Mouse or Human Cells

[H], human; iPSC, induced pluripotent stem cell; K, Klf4; L, Lin-28; M, c-Myc; [M], mouse; N, Nanog; NaB, sodium butyrate; NP, neural progenitor; NSC, neural stem cell; O, Oct-4; S, Sox2; UTF, undifferentiated cell transcription factor.

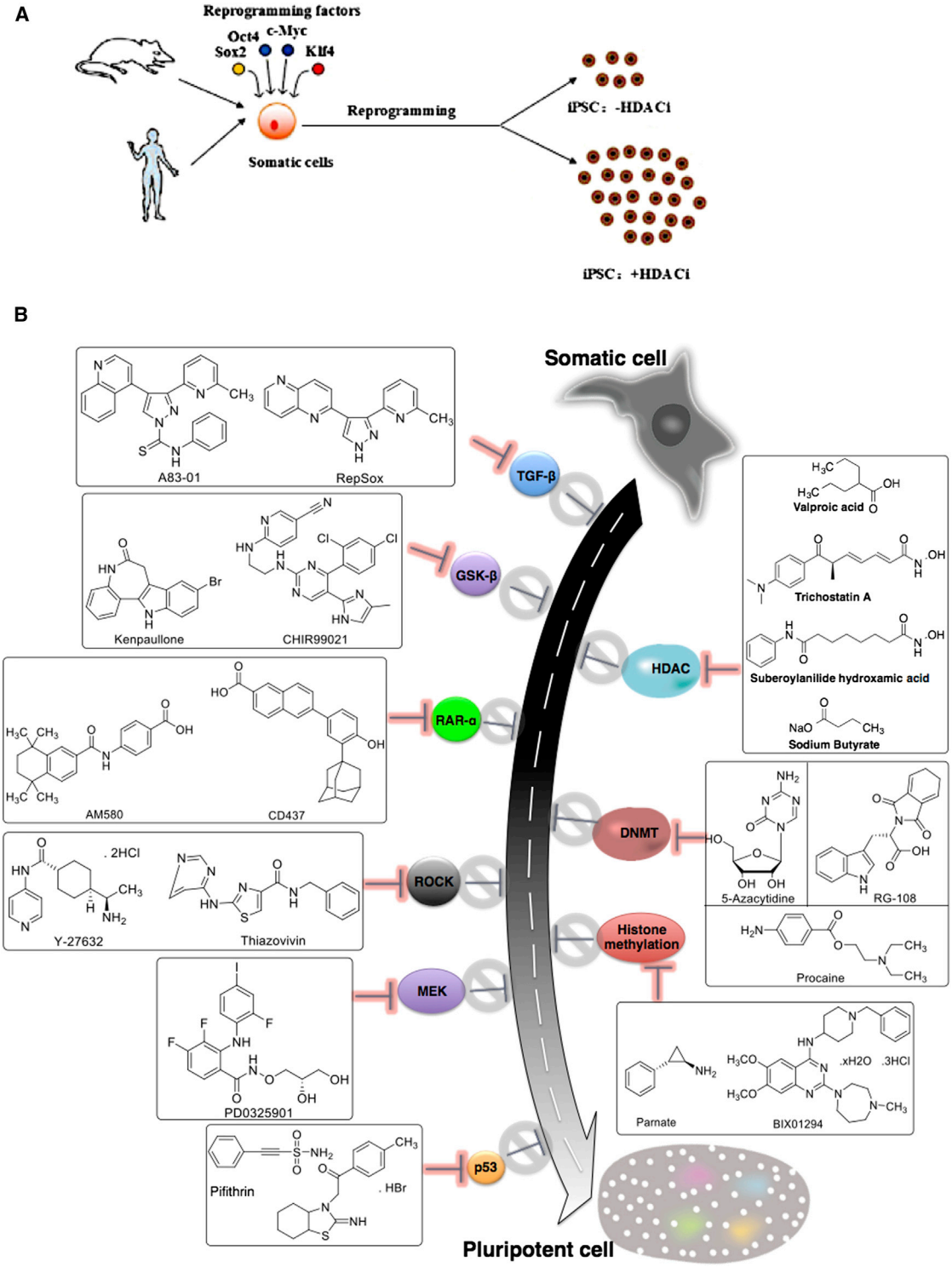


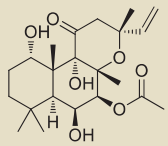
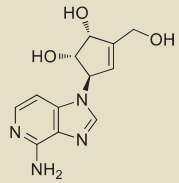
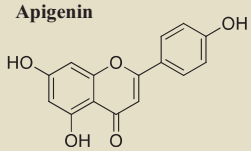
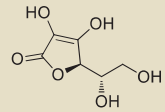
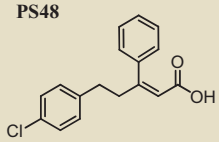
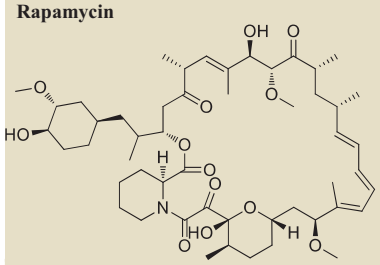
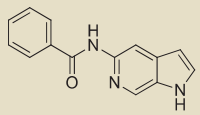
Figure 2. Small Molecules with Reprograming Effects

(A) Histone deacetylase (HDAC) enhances reprogramming efficiency. The efficiency of reprogramming of somatic cells into the pluripotent state using the four transcription factors improves by more than 100-fold with HDAC inhibitor (HDACi) especially valproic acid than that without HDACi.

(B) Small molecule inhibitors for factors blocking the conversion of somatic cells to pluripotent stem cells. Some commercially available small molecules known to improve reprogramming efficiency by inhibiting signaling pathways, such as transforming growth factor TGF-β, GSK-β, MEK, RAR-α, ROCK, p53 (Feng et al., 2008; Kawamura et al., 2009; Wang et al., 2011b), and chromatin-modifying enzymes, such as histone methylases, HDACs, and DNA methyltransferases (DNMTs), are shown.

GSK-β, glycogen synthase kinase 3β; LSD, lysine-specific demethylase; MEK, mitogen-activated protein kinase kinase; PDK, protein kinase; RAR, retinoic acid receptor; ROCK, Rho-associated kinase; TGF-β, transforming growth factor β.

Table 2. Chemicals Harnessed for the Generation of iPS Cells

Chemical	Function	Cells	Reference
Forskolin 	Diterpene activator of adenylate cyclase	Human neural stem cells	(Hester et al., 2009)
DZNeP 	Global histone methylation inhibitor	MEF	(Hou et al., 2013)
Apigenin 	Downregulation of a H3K4-specific histone methylase	MEF	(Chen et al., 2010)
Vitamin C 	Enhances the generation of mouse and human iPSC	MEF, HDF	(Esteban et al., 2010)
PS48 	Phosphoinositide-dependent protein kinase-1 activator	Primary NHEK	(Zhu et al., 2010)
Rapamycin 	Mammalian target of rapamycin inhibitor	MEF	(Chen et al., 2011)
Lithium	Downregulation of a H3K4-specific histone methylase	MEF, HUVEC	(Wang et al., 2011a)
Oct-4 activator (OAC1) 	Inhibition of the p53-p21 pathway or activation of the Wnt-β-catenin signaling.	MEF	(Li et al., 2012)
NaCl	Hyper osmosis and reduction of global demethylation level	MEF	(Xu et al., 2013)

DZNeP, 3-deazaneplanocin; H3K4, histone H3 lysine 4; HDF, human dermal fibroblast; HUVEC, human umbilical vein endothelial cell; MEF, mouse embryonic fibroblast; NHEK, normal human epidermal keratinocyte.

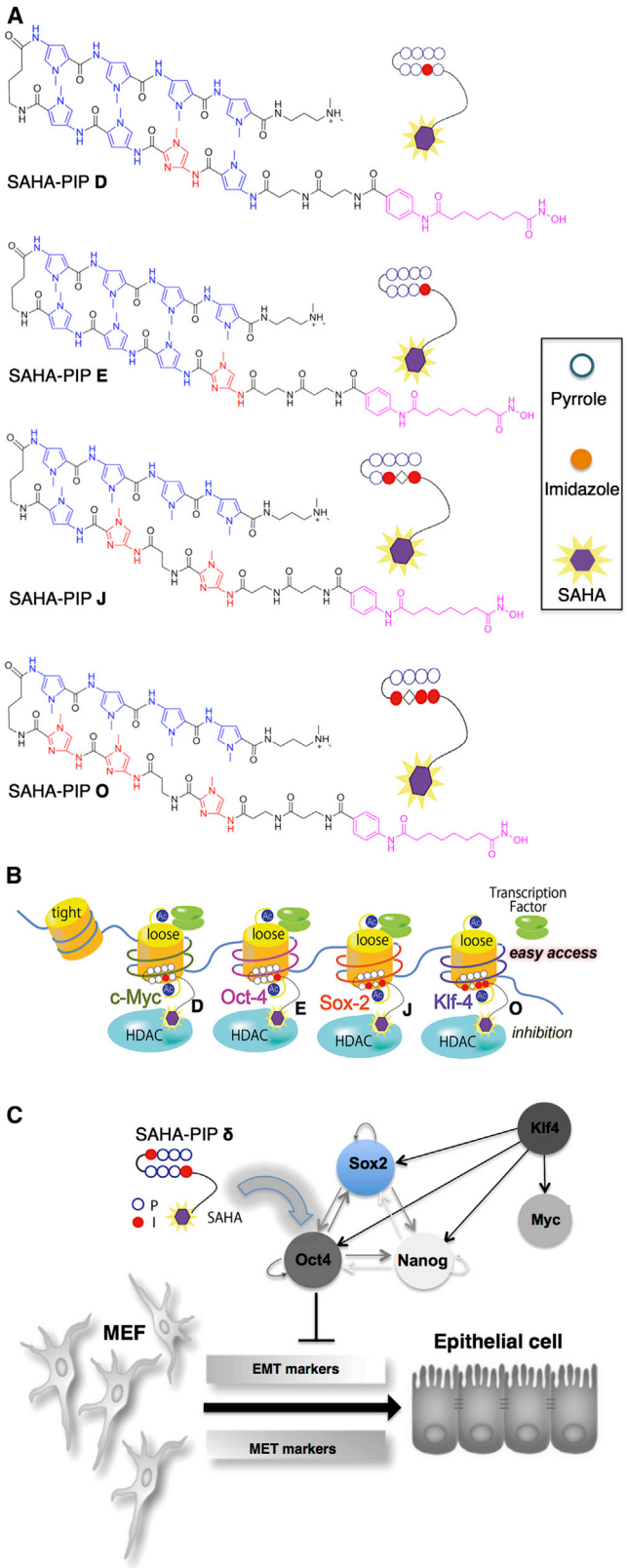


Figure 3. Suberoylanilide Hydroxamic Pyrrole-Imidazole Polyamides and Pluripotency Genes

(A) Chemical structures of the suberoylanilide hydroxamic pyrrole-imidazole polyamides SAHA-PIP D, -E, -J, and -O and their simplified illustrations.

(B) SAHA-PIPs induce site-specific acetylation, which in turn triggers the transcription of *c-Myc* (D), *Oct-3/4* (E), *Sox2* (J), and *Klf4* (O).

(C) SAHA-PIP δ triggers the core pluripotency gene network, but not Klf4, to initiate cellular reprogramming via the downregulation of the mesenchymal markers and the upregulation of epithelial markers (modified version of Pandian et al., 2012a).

Programmed Multitarget Molecules for Cellular Reprogramming: Current Progress and Future Challenges

Generation of iPS cells depends upon the concomitant expression of multiple factors; thus, many small molecules might be needed to achieve reprogramming using chemicals. Development of innovative small molecules that have one or more functional activities is an alternative option. Hairpin pyrrole–imidazole polyamides (PIPs) are programmable synthetic molecules that can bind to the minor groove of DNA with an affinity that is similar to that of natural transcription factors (Kashiwazaki et al., 2012; Pandian and Sugiyama, 2012, 2013). PIPs are versatile small molecules and could be developed for use in many applications, as they have flexible sites for covalent attachment to molecules, such as fluorescent dyes and/or some enzyme inhibitors (Pandian and Sugiyama, 2012; Vaijayanthi et al., 2012; Vaijayanthi et al., 2013).

In 2011, the Sugiyama group achieved a unique breakthrough by synthesizing a small-molecule class, called *SAHA-PIP*, by conjugating PIP with SAHA. As mentioned earlier, SAHA has potent inhibitory activity against HDAC1, the chromatin-modifying enzyme that regulates pluripotency and lineage-specific transcriptional networks (Huangfu et al., 2008; Kidder and Palmer, 2012). Hence, a series of 16 SAHA-PIPs that could cause differential gene activation were screened to identify the bioactive SAHA-PIPs that can modulate the endogenous expression of standard reprogramming factors in MEFs. Interestingly, 4 of the 16 SAHA-PIPs (D, E, J, and O; Figure 3) distinctively induced the expression of *Oct-3/4*, *Nanog*, *Sox2*, *Klf4*, and *c-Myc* (Pandian et al., 2011). SAHA-PIP-mediated gene induction occurred by establishing transcriptionally permissive chromatin, including Lys9 and Lys14 acetylation and Lys4 trimethylation of histone H3. Modifications of the chemical architecture of one the SAHA-PIPs (Figure 3E), which induced Oct-3/4 and Nanog expression, resulted in the improvement of expression levels; however, the effect was only marginal (Pandian et al., 2012b). Nevertheless, the scope of the improvement demonstrated in this work confirmed the possibility of tailoring SAHA-PIPs and improving their efficacy. Subsequently, a novel library of SAHA-PIPs with improved recognition of GC-rich sequences revealed a potent SAHA-PIP that can induce multiple pluripotency genes in just 24 hr and at nanomolar concentrations. A microarray analysis revealed that this SAHA-PIP, designated "δ," switched the transcriptional network from the fibroblast state to the pluripotent state. This novel small molecule also rapidly overcame the mesenchymal–epithelial transition (MET) stage, which is an important rate-limiting step during the dedifferentiation of the somatic genome (Pandian et al., 2012a; Polo and Hochedlinger, 2010). More details of certain SAHA-PIPs and their target pluripotency genes are shown in Figure 3.

As the clinical utility of iPS cells improves with the decrease in the number of reprogramming factors, the identification of

programmable SAHA-PIPs that could induce multiple pluripotency genes may lead to the efficient generation of pluripotent stem cells. A recent report showed that subtle modification in the structure of SAHA could alter the specificity of SAHA-PIP δ toward different HDAC enzymes (Saha et al., 2013). A systematic approach is thus in demand to mimic the natural cellular environment and design preprogrammed chromatin-remodeling PIPs to bypass the conventional routes to achieving pluripotency. Although the concept of a small-molecule multifactor is promising, several bottlenecks, such as cell permeability issues related to small molecules, as well as the identification of appropriate cell sources, are essential because the epigenome of each cell type is different.

Essential Role of Optimal Cell Sources for the Efficient Generation of CGiPS Cells

The point from which one starts defines the time and the way in which one needs to reach a destination. From this perspective, not only the route of reprogramming, but also the choice of cell sources have a significant impact on the probability of reaching the desired destination of clinically useful iPS cells. Furthermore, the use of less-invasive methods to obtain source cells can generate readily available, larger amounts of cells (Ohmine et al., 2012). Accordingly, human keratinocytes are a good source from which to derive iPS cells with high efficiency, as they can be obtained by simply plucking a patient's hair (Aasen et al., 2008). Gingival fibroblasts (GFs) are the main components of the gingival connective tissues and play a cardinal role in oral wound-healing. Moreover, their culture is also relatively simple, as GFs adhere well, spread onto the culture plates, and proliferate rapidly, even under ordinary culture conditions (Egusa et al., 2010). Hence, GFs are an ideal source for (1) the generation of iPS cells that can be translated clinically in dentistry and (2) the establishment of an iPS cell bank that could be harnessed for comprehensive medical applications. One of the barriers that hinders the clinical translation of iPS cells is the need to xenoculture cells on feeder layers. Anchan and colleagues generated iPS cells within 5–7 days from mouse and human amniocytes using retroviral vectors encoding either one of the four factors or two factors, and pluripotency could be maintained over mitotically inactivated amniocytes. Interestingly, the efficiency of iPS cell generation from amniocytes was about 10-fold higher than that observed for iPS cells generated from MEFs or human dermal fibroblasts (Anchan et al., 2011). With the derivation of cellular components from a single donor, the safety concerns associated with xenoculture can be annulled. Only two factors (Klf4 and c-Myc) were sufficient to achieve amniocyte-derived iPS cells that could be differentiated into the three germ layers.

Panopoulos and colleagues reported that iPS cells could be achieved rapidly (in just 6 days) from HUVECs with greater efficiency (approximately 300-fold higher) compared to human fibroblasts (Panopoulos et al., 2011). Reprogramming of HUVECs under hypoxic conditions and in the presence of a TGF-β inhibitor had better efficiency (about 2.5-fold) than those reprogrammed under standard conditions. HUVECs are also an attractive somatic source for therapeutic applications. Likewise, iPS cells generated from human cord blood (Giorgetti et al., 2009) could be a stored and readily available human leukocyte antigen–type somatic cell. The adipose tissue that is available abundantly in the body is another preferred cell source from which to derive iPS cells. Human and mouse adipose-derived stem (ADS) cells were reprogrammed into a pluripotent state under standard feeder-dependent conditions with high efficiency until the 1.5% level was reached (Sugii et al., 2011). ADS cells can be developed for therapeutic use, as they proliferate rapidly and differentiate into adipocytes, osteocytes, chondrocytes, and myocytes (Gimble et al., 2007). Employment of cord blood cells, HUVECs, or human amniocytes as starting cells for reprogramming also has limitations. These cells are not always available for patients and individuals who manifest certain disease symptoms and demand the use of personalized iPS cell–based regenerative therapy. Recently, Wang and colleagues reprogrammed epithelial-like human urine cells into neural progenitors using episomal vectors (Wang et al., 2013).

Journey toward CGiPS Cells and Major Roadblocks

An increasing number of studies in mouse models suggest the therapeutic potential of iPS cells. One such study showed that when iPS-derived neurospheres preevaluated as "safe" are transplanted into a severe combined immunodeficiency mouse brain, they produce functional nerve cells under in vitro conditions with no tumor formation (Tsuji et al., 2011). Such safe iPS-cell-derived cells should be employed for transplantation therapies. Ye and colleagues successfully reprogrammed skin fibroblasts from a homozygous β-thalassemia patient into pluripotent cells, and the derived iPS cells differentiated into hematopoietic cells that synthesized hemoglobin (Ye et al., 2009). In some countries, prenatal diagnosis and selective abortion reduce the number of β-thalassemia births. Early treatment is preferable to adult treatment because the former requires fewer cells than the latter. Hence, iPS cell therapy may contribute to effective treatment of this disease in the perinatal period. Generation of iPS cell lines from human Fanconi anemia (FA) patients and gene-corrected FA-iPS cell lines demonstrated the possibility of acquiring disease-corrected, patient-specific cells (Raya et al., 2009).

Retinal cells are the most accessible part of the central nervous system and provide an ideal platform for the evaluation of the therapeutic potential of iPS cells in clinical settings. Human iPS cells can be maintained and directed to differentiate into retinal cell types under xeno-free conditions (Sridhar et al., 2013). Retinal cells rarely develop cancers, and, even if a tumor arises, it can easily be removed with a laser-based procedure. Taking these advantages into consideration, Masayo Takahashi has announced a clinical trial to treat age-related macular degeneration, which is a common cause of blindness that affects at least 1% of people older than 50 years of age (http://www.riken.jp/en/pr/press/2013/20130730_1/; see also Cyranoski, 2013). Researchers hope that this trial is successful, as it will show that iPS cells could be used in the clinic.

Using mouse cells, Zhao and colleagues demonstrated the iPS-mediated induction of T cell–dependent immune response in syngeneic recipients, suggesting the requirement of careful validation before clinical trials (Zhao et al., 2011). In contrast, Araki and colleagues showed that in regressing skin and teratoma tissues of mice, transplanted cells differentiated from iPS cells and that ESCs triggered a limited or no immune response

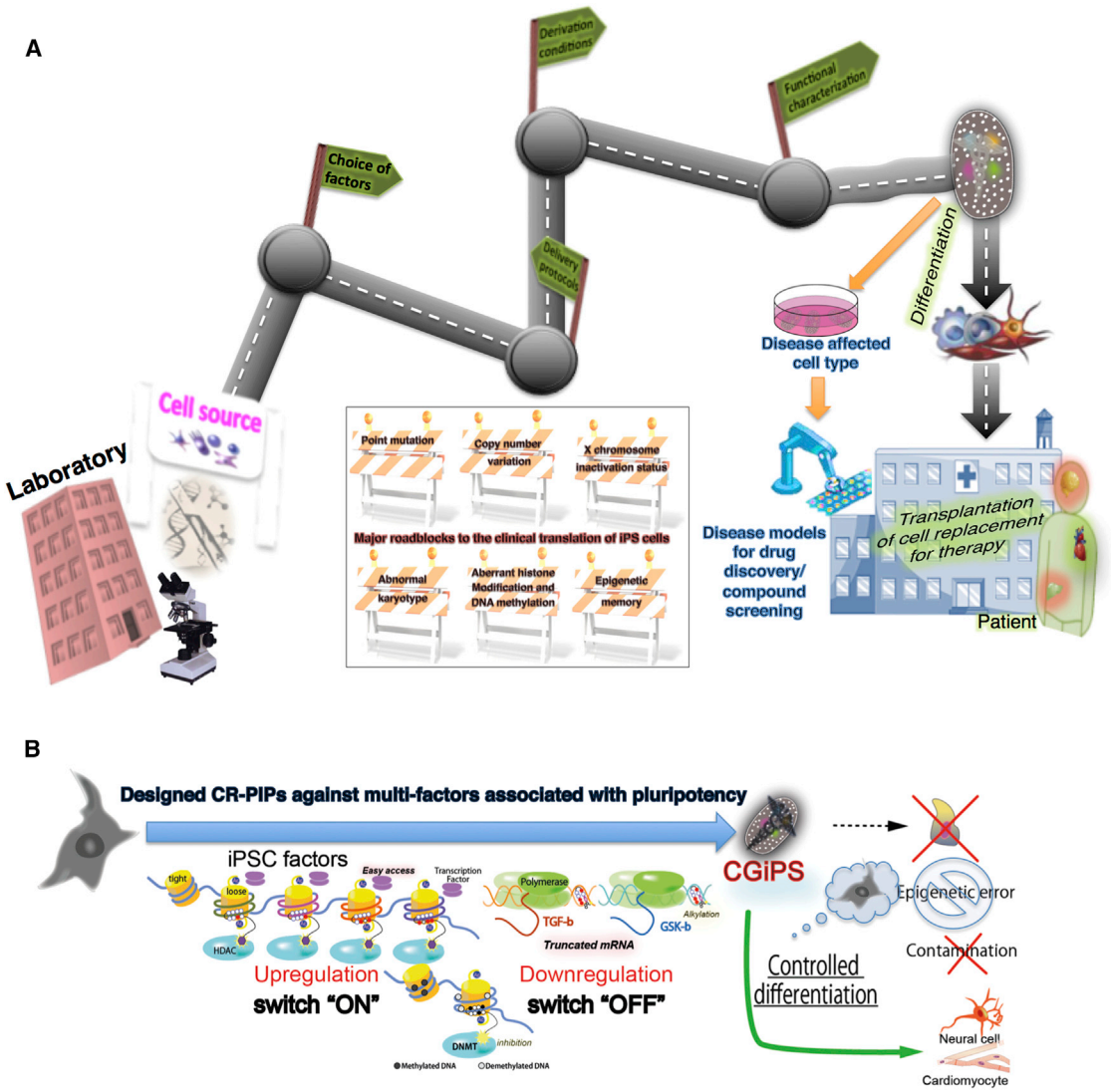


Figure 4. Potential of Chromatin-Remodeling Pyrrole-Imidazole Polyamides to Generate CGiPS Cells

(A) Several deviations exist in the conventional route to switch laboratory-grade iPS cells to CGiPS cells. In the box, some major drawbacks get illustrated as roadblocks as in Barrilleaux and Knoepfler (2011).

(B) The development of multifactor-targeting small molecules, such as chromatin-remodeling pyrrole–imidazole polyamide (PIP), that could selectively and concomitantly switch the essential genes/factors "ON" and "OFF" may lead to the generation of CGiPS cells.

(Araki et al., 2013). The genomic integrity of iPS cells is another concern, as iPS cells have more copy number variations (CNVs) in their genomes than ESCs or their parental fibroblasts in human cells (Hussein et al., 2011). However, Abyzov and colleagues implied that reprogramming does not necessarily lead to de novo CNVs in iPS cells, because most of the line-manifested CNVs reflect somatic mosaicism in the human skin (Abyzov et al., 2012). Hence, iPS cells could be as useful as ESCs in human cells.

Conclusions

Development of tailor-made therapeutics is the ultimate goal of the use of iPS cells. In this context, small molecules have the advantage, as they can be designed and optimized according to the patient's needs. Small molecules have shown success in obviating the need for retroviruses, and complete chemical reprogramming has now been achieved in mouse cells. However, at least one transcription factor, such as OCT4, is essential for the generation of iPS cells using human cells. Although small molecules increase reprogramming efficiency, the overall efficiency of this approach remains lower than that observed with the use of mRNA-based induction protocols. The populations of nonresponsive or stochastically resistant fibroblasts may outnumber those that are responsive to the reprogramming protocol. Some other unknown factors could also play a key role in improving the efficiency of reprogramming. Therefore, the development of different types of multitarget small molecules, such as SAHA-PIP, that could rapidly induce multiple pluripotency in somatic cells via site-specific epigenetic activation is relevant, as the genomewide gene expression profile

and acetylation pattern of SAHA-PIP-treated cells were comparable to those seen in ESCs (Pandian et al., 2012a). Epigenetic reprogramming could also be the key to improving the clinical utility of iPS cells (Fisher and Merkenschlager, 2010). Studies in human cells suggested that significant regions of the iPS epigenome do not revert to the embryonic state; rather, they retain the epigenetic memory of their tissue of origin (Lister et al., 2011). Chromatin-modifying enzymes could act as both facilitators of and barriers to the epigenetic remodeling of differentiated cells into a stem cell configuration in mouse and human cells (Hirai et al., 2011; Onder et al., 2012). Therefore, small molecules, such as SAHA-PIPs, that can induce sequence-specific chromatin modifications (Han et al., 2013) need to be developed to erase the epigenetic memory and aid the generation of CGiPS cells. Although SAHA-PIP research is in its primitive stage, the development of such innovative small-molecule-based strategies may offer new opportunities to transfer the iPS cell technology from the laboratory to the clinic as shown in Figure 4.

Identification of a small-molecule, pluripotent cell-specific inhibitor (PluriSIn#1; *N′*-phenylisonicotinohydrazide) that selectively eliminated human pluripotent cells substantiated the opportunities to develop demand-based small molecules via screening (Ben-David et al., 2013). Although exciting advancements in developing chemical biology tools are taking place at an astonishing pace, careful validation is needed to minimize safety concerns. Nevertheless, innovative strategies and optimal cell sources will likely allow the efficient generation of CGiPS cells in the near future.

ACKNOWLEDGMENTS

We gratefully acknowledge the financial support from the Science Foundation of Health Ministry (WKJ2009-2-019) and the Public Technology Research and Social Development Project of Zhejiang Province (20011C23004). We acknowledge the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan. We thank the iCeMS exploratory grant and Grants-in-Aid for Young Scientists (B) for support provided to G.N.P.

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